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Effectiveness of a novel cellular therapy to treat multidrug-resistant tuberculosis

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ABSTRACT

Introduction: We urgently need novel treatments for multidrug-resistant tuberculosis (MDR-TB). Autologous mesenchymal stromal cell (MSC) infusion is one such possibility due to its potential to repair damaged lung tissue and boost immune responses. We aimed to assess the effectiveness of MSC to improve outcomes among MDR-TB patients.

Methods: We analyzed outcomes for 108 Belarussian MDR-TB patients receiving chemotherapy. Thirty-six patients ("cases") also had MSCs extracted, cultured and re-infused (average time from chemotherapy start to infusion was 49 days); another 36 patients were "study controls". We identified another control group: 36 patients from the Belarussian surveillance database ("surveillance controls") 1:1 matched to cases.

Results: Of the cases, 81% had successful outcomes versus 42% of surveillance controls and 39% of study controls. Successful outcome odds were 6.5 (95% Confidence Interval: 1.2–36.2, $p = 0.032$) times greater for cases than surveillance controls (age-adjusted). Radiological improvement was more likely in cases than study controls. Culture analysis prior to infusion demonstrated a poorer initial prognosis in cases, yet despite this they had better outcomes than the control groups.

Conclusion: MSC treatment could vastly improve outcomes for MDR-TB patients. Our findings could revolutionize therapy options and have strong implications for future directions of MDR-TB therapy research.

1. Introduction

Despite recent reductions in tuberculosis (TB) incidence and mortality [1], slow progress is threatened by emerging drug resistant strains, responsible for 480,000 multidrug-resistant TB (MDR-TB; TB strains resistant to isoniazid and rifampin) cases in 2013 [1]. Current MDR-TB drugs are more toxic and have to be taken for longer than those for drug susceptible TB [2]; successful outcome rates are poorer [3] and only around half of treated MDR-TB cases globally are cured or complete treatment successfully [1,4]. Currently, the development of anti-tuberculosis drugs lags behind that of *Mycobacterium tuberculosis* drug resistance. We urgently need novel treatment options to improve outcomes for MDR-TB cases [5].

Belarus, in Eastern Europe, has the highest reported percentage of TB cases with MDR-TB in the world (45.5% of all TB cases in Belarus have MDR-TB) [6] and less than 50% of these patients are treated successfully (as per the World Health Organization [WHO] definition, treated successfully includes those cured and completed treatment with no evidence of failure of treatment) [4]. In 2009,

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a pilot study began in Belarus to assess the safety and effectiveness of autologous mesenchymal stromal cell (MSC) infusion as adjunct treatment in MDR-TB patients [7]. Although host-directed therapies have been hailed as a breakthrough for cancer [8], new concepts and clinically relevant trials are needed to achieve similar life-changing progress in infectious diseases.

The study motivation came from evidence that MSCs can facilitate organ homoeostasis and repair damaged lung tissue [9]. Additionally, it is possible that immunotherapeutic methods could reduce high inflammatory immune response in TB [10] with MSCs being one such potential method [11]. In the pilot study, the MSC treatment was found to be safe [7]. Here, we use these Phase I trial data to obtain a preliminary assessment of the MSC treatment effectiveness and give an indication of the potential value of Phase II/III trials.

2. Methods

We conducted an observational study using (1) outcome data from a non-randomised controlled trial in Belarus, and (2) data collected from the Belarussian surveillance database.

2.1. Patient recruitment to the trial

An ongoing open-label phase I non-randomized controlled trial of MSC infusion as adjunct treatment in MDR-TB patients has been conducted since September 2009 at the Republican Research and Practical Centre for Pulmonology and TB (RRPCPTB), Minsk, Belarus [7]. This includes those with extensively drug resistant TB (XDR-TB; MDR-TB plus resistance to an injectable drug and a fluoroquinolone), those with "pre-XDR-TB" (MDR-TB plus resistance to either an injectable or a fluoroquinolone) and MDR-TB patients without additional resistance. Seventy-two patients have been recruited: 36 that agreed to receive the MSC therapy ("cases") and 36 that did not agree to the treatment but consented to the monitoring necessary for the study ("study controls"). The main inclusion criteria were pulmonary TB confirmed by culture; MDR-, pre-XDR- or XDR-TB confirmed by drug susceptibility testing; age between 18 and 65 years; and absence of lesion compatible with a malignant process or ongoing tuberculosis in organs other than lungs and pleura [7]. Individuals with the following co-morbidities were also excluded: HIV, hepatitis B and/or C, autoimmune diseases, multi-organ failure, sepsis (any bacterial sepsis), abscess formation other than TB etiology, cancer and other malignancies, anti-DNA antibodies, allergies and any other disease that researchers believed was clinically significant and could affect the study results or cause an additional risk to the patient. Participants were not compensated for taking part in the study or for their travel expenses. Full details of this study are available elsewhere [7] and in the Appendix.

2.2. Selection of alternative controls from surveillance data

Since the study was non-randomized, there was potential for differences between cases and study controls that could have influenced the apparent MSC treatment effectiveness. Therefore, for a parallel analysis we selected matched controls ("surveillance controls") from the Belarussian TB surveillance database. Briefly, this database contains all reported TB cases in Belarus since 2009 and their demographic and clinical data. We 1:1 matched cases to controls (who met the original study inclusion criteria [7]) from the surveillance database on: (1) drug resistance profile (MDR-TB, pre-XDR-TB or XDR-TB), (2) previous TB treatment history (<one month of treatment ("new") or ≥one month of treatment ("previously treated")) and (3) baseline smear microscopy status (positive/negative). Previously treated patients in the trial were further stratified into "previously treated" (previous treatment(s) with first line drugs and/or less than two treatments with second line drugs) and "chronic" (≥2 previous second line regimens) but surveillance controls were not stratified in this way, due to lack of information regarding the number of times they had previously received treatment. Matching criteria were selected after reviewing the baseline differences between cases and study controls. We randomly selected surveillance controls although patients from Minsk city were prioritised due to logistical difficulties elsewhere in obtaining matching and potential confounder data.

2.3. Treatment and monitoring

All patients received an individualized optimal background regimen throughout their treatment period in accordance with WHO guidelines [12] (Appendix Table S1-3). In addition, bone marrow aspirates of 40–80 mL were obtained from the iliac crest of the cases, MSCs were isolated, cultured, prepared for infusion and reinfused on average 49 days after chemotherapy initiation, as described previously [7]. The entire MSC cell dose was given as a slow (5 min) bolus injection via a peripheral intravenous line.

For cases and study controls, chest X-rays were taken at chemotherapy initiation and approximately eight months later to assess changes. X-rays were assessed by experienced radiologists, who were blind to treatment, and scored as per Ralph et al. [13] (briefly, the score equals the percentage of lung involvement, plus 40 if cavities are present). Adverse event information among cases and study controls was collected during the first six months after MSC infusion and six months corresponding period (starting one month after chemotherapy initiation) for the study controls.

Microbiological data were collected from patient medical records or from reporting systems for National TB control program. The RRPCPTB received External Quality Assurance from the Swedish Institute for Infectious Disease Control throughout the study period. TB was confirmed with direct microscopy after Ziehl–Neelsen staining and culture and drug susceptibility testing were done using the BACTEC MGIT 960 system (Becton Dickinson, Sparks, MD, USA).

2.4. Baseline characteristics

We compared the baseline characteristics of cases with each control group using a *t*-test (for continuous variables) or Fisher's exact test (for categorical variables). Since the patient's choice to receive the MSC treatment may have been associated with hard to quantify factors such as motivation to be cured or general knowledge about and desire for optimal health, we examined potential proxies for these factors (smoking, employment, education and marital status).

2.5. Analysis of outcomes

All outcome definitions were consistent with the 2008 WHO guidelines [12] for MDR-TB patients (2008 guidelines used because the original trial began in 2009).

Successful outcomes included "cured" and "treatment completed" and all other outcomes were "unsuccessful" (e.g. death, treatment failure, treatment default/lost to follow-up). "Cured" is defined as treatment completed as recommended by the national policy without evidence of failure and five or more consecutive cultures taken at least 30 days apart negative in the final 12 months of treatment. "Treatment completed" is defined as treatment completed as recommended by the national policy without evidence of failure but with fewer than five cultures performed in the final 12 months of treatment. Both of these categories consist

Table 1
Baseline characteristics. Baseline characteristics of patients that received the MSC treatment ("cases"), controls selected for the study ("study controls") and controls selected from surveillance data ("surveillance controls").

Variable	Cases ($N=36$)	Study controls ($N=36$)	Surveillance controls ($N=36$)	p-Value for difference (cases versus study controls	p-Value for difference (cases versus surveillance controls)
Age, mean (SD)	30.5 (8.5)	38.8 (13.9)	44.1 (10.9)	0.004	<0.0001
Gender, *n*, (% male)	18 (50.0%)	25 (69.4%)	31 (86.1%)	0.15	0.002
Treatment history					
New case, *n* (%)	13 (36.1%)	19 (52.8%)	13 (36.1%)	0.077	1.00[a]
Previously treated case, *n* (%)	11 (30.6%)	13 (36.1%)	23 (63.9%)[b]		
Chronic case, *n* (%)	12 (33.3%)	4 (11.1%)			
Drug resistance profile					
MDR, *n* (%)	9 (25.0%)	20 (55.6%)	9 (25.0%)		
Pre-XDR, *n* (%)	12 (33.3%)	10 (27.8%)	12 (33.3%)	0.020	1.00[a]
XDR, *n* (%)	15 (41.7%)	6 (16.7%)	15 (41.7%)		
Smear positive at start of chemotherapy, *n* (%)	5 (13.9%)	18 (50.0%)	5 (13.9%)	0.002	1.00[a]
Current smoker, *n* (%)	20 (55.6%)	25 (69.4%)	26 (72.2%)	0.33	0.22
Employment status					
Unemployed, *n* (%)	5 (13.9%)	13 (36.1%)	3 (8.3%)		
Employed, *n* (%)	18 (50.0%)	13 (36.1%)	23 (63.9%)		
On disability benefits, *n* (%)	6 (16.7%)	4 (11.1%)	7 (19.4%)	0.030	0.11
Student, *n* (%)	5 (13.9%)	1 (2.8%)	0 (0.0%)		
Maternity leave/housewife, *n* (%)	2 (5.6%)	1 (2.8%)	1 (2.8%)		
Retired, *n* (%)	0 (0.0%)	4 (11.1%)	2 (0.0%)		
Marital status					
Single, *n* (%)	17 (47.2%)	20 (55.6%)	16 (44.4%)		
Married, *n* (%)	18 (50.0%)	13 (36.1%)	16 (44.4%)	0.49	0.50
Divorced, *n* (%)	1 (2.8%)	2 (5.6%)	4 (11.1%)		
Widowed, *n* (%)	0 (0.0%)	1 (2.8%)	0 (0.0%)		
Highest education level					
Secondary school, *n* (%)	9 (25.0%)	11 (30.6%)	5 (13.9%)		
College, *n* (%)	17 (47.2%)	17 (47.2%)	22 (61.1%)		
Currently at university, *n* (%)	5 (13.9%)	1 (2.8%)	0 (0.0%)	0.060	0.040
University, *n* (%)	5 (13.9%)	2 (5.6%)	9 (25.0%)		
No data, *n* (%)	0 (0.0%)	5 (13.9%)	0 (0.0%)		

[a] Controls were matched to MSC patients on these variables.
[b] Chronic and previously treated patients were all grouped as previously treated patients in the surveillance database.

of patients that are clinically cured of TB, the difference is in the degree of bacteriological evidence.

We examined the percentage of cases and each control group that had a successful outcome within each stratum of the baseline characteristics to check for consistency in outcomes rates across strata. When comparing outcomes in cases and study controls, we used logistic regression to model the odds of a successful outcome and a conditional logistic regression when comparing cases to surveillance controls to account for the 1:1 matching. All potential explanatory variables were entered into the model as categorical variables, other than age, which was entered as a continuous variable. In both, a binary, explanatory variable defined case/control status of the patient. Due to the relatively small patient numbers and resulting potential for model instability, we could not adjust for all potential confounders simultaneously. Since we aimed to produce MSC treatment effect estimates, we produced univariable regression estimates of the odds of a successful outcome among cases versus controls and a series of bivariable regression models to assess the effect of each potential confounder on the treatment estimate.

As a sub-analysis, we used sputum culture conversion as an alternative endpoint (Appendix methods).

2.6. Percentage of patients that were culture positive at two, four and six months

We calculated the number and percentage of patients in each group whose sputum converted to culture negative status after two, four and six months of treatment. We compared the differences between these percentages in each group, at each time point, using Fisher's exact test.

2.7. Analysis of radiology

The percentage change in X-ray score was calculated and classed as improved (≥10% decrease), worsened (≥5% increase) or stable otherwise [7]. We compared the number of cases and study controls that had an improved or worsened radiology score at follow-up using Fisher's exact test. Radiology data were not available for surveillance controls.

2.8. Adverse events

We compared the number of cases and study controls that experienced each adverse event using Fisher's exact test, adjusting for multiple testing using a Bonferroni correction [14] (see Appendix). Information on adverse events was not available for the surveillance controls.

Statistical analyses of non-identifiable data from the described datasets were deemed exempt by Partners Healthcare Research Committee (the IRB for Partners Healthcare on behalf of Brigham and Women's Hospital), Boston, MA. HEJ carried out all statistical analyses.

3. Results

3.1. Comparison of baseline characteristics

Cases and study controls differed substantially at baseline. Cases were more likely to be chronic than new patients compared to study controls ($p=0.07$) and were more likely to have pre-XDR-TB or XDR-TB ($p=0.02$) (Table 1). Cases were less likely to be smear microscopy positive (13.9% versus 50.0%; $p=0.002$). Study

Table 2
Outcomes for MSC recipients (cases), study controls and matched surveillance controls.

	Cases ($n = 36$)	Study controls ($n = 36$)	Surveillance controls ($n = 36$)
Successful outcomes:	29 (81%)	14 (39%)	15 (42%)
Cured	27 (75%)	8 (22%)	8 (22%)
Treatment completed	2 (6%)	6 (17%)	7 (19%)
Unsuccessful outcomes:	7 (19%)	22 (61%)	21 (58%)
Death	0 (0%)	0 (0%)	4 (11%)
Default/lost to follow-up	1 (3%)	6 (17%)	5 (14%)
Treatment failure	6 (17%)	16 (44%)	12 (33%)

controls were more likely to be unemployed ($p = 0.03$) and less likely to have attained university level education ($p = 0.06$).

Surveillance controls were, on average, older than cases (mean age = 44.1 years versus 38.8 years, $p < 0.0001$) and were more likely to be male (86.1% male versus 50.0%, $p = 0.002$) (Table 1). Surveillance controls were more likely than cases to have attained university level education ($p = 0.04$) and were somewhat less likely to be unemployed ($p = 0.11$).

3.2. Analysis of outcomes

Among cases, 29 (81%) patients had a successful outcome (Table 2). Six (17%) patients failed treatment and one (3%) defaulted on treatment. Among the study controls, 14 (39%) patients had a successful outcome. Sixteen (44%) failed treatment and six (17%) defaulted or were lost to follow up. Among the surveillance controls, 15 (42%) had a successful outcome, four (11%) died, five (14%) defaulted or were lost to follow up and 12 (33%) failed treatment.

The percentage of outcomes that was successful among cases was consistently higher than among either of the control groups, across almost all strata of the baseline characteristics (Table 3). The exceptions were chronic TB patients and those on disability (two almost entirely overlapping groups).

When assessing successful outcome odds, we used the comparison with the surveillance controls as our main analysis (since they were more similar to cases at baseline than the study controls). After adjusting for age (the only negative confounder of those we could assess), cases had odds of a successful outcome 6.5 (95% Confidence Interval: 1.2–36.2) times greater than the surveillance controls ($p = 0.032$) (Table 4).

Results of the comparison with study controls and sputum culture conversion analysis supported these results (Appendix Tables S4 and S5).

3.3. Culture negative status at two, four and six months

Two months after chemotherapy start, fewer cases had converted to culture negative status than study controls (5.7% versus 27.3%, p-Value = 0.053) or surveillance controls (5.7% versus 38.9%, p-Value = 0.010) (Fig. 1). At four months, 60.6% of cases had culture converted compared with 43.8% of the study controls (four month data were unavailable for surveillance controls). At six months, 71.9% of cases had converted compared with 58.6% of the study controls and 71.4% of the surveillance controls (p-Values both > 0.05 for differences).

3.4. Analysis of radiology

Twenty-five cases showed improvement in their radiology score compared with 15 study controls ($p = 0.032$ for the difference). Four cases had a worsened radiology score compared with nine study controls ($p = 0.22$).

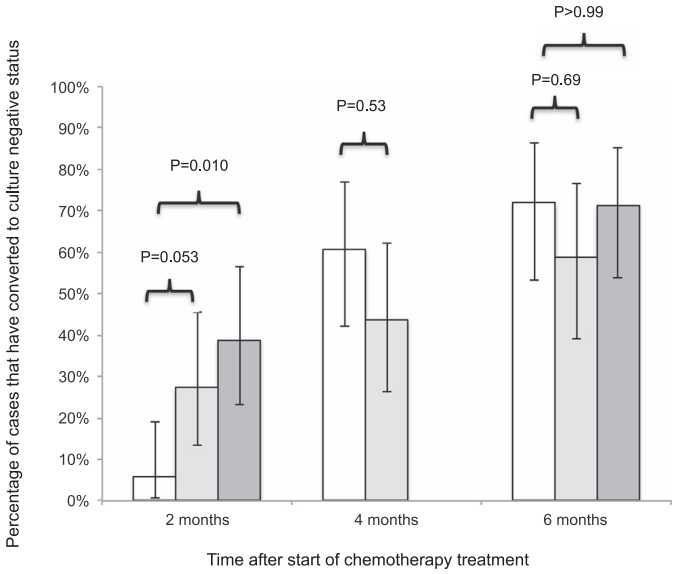

Fig. 1. *Sputum culture conversion during treatment*. The percentage of patients that converted to culture negative status at two, four and six months after start of chemotherapy treatment. Data are shown for cases (white bars), study controls (pale grey bars) and surveillance controls (dark grey bars). Data at four months were not available for the surveillance controls. p-Values shown are for the differences between the cases and each of the control groups at each time point for which data were available.

3.5. Adverse events

The most common adverse events among cases were hypercholesterolaemia and nausea (Appendix Table S6). There were no significant differences in the adverse event rate between cases and study controls.

4. Discussion

This study is, to our knowledge, the first to estimate the efficacy of any cellular therapy for M/XDR-TB. With 81% of MSC recipients experiencing a successful outcome as compared with 39% or 42% in the control groups, the effect appears striking.

Several biological mechanisms can explain the improved outcomes observed. The MSC clinical efficacy can be explained by a reduction of inflammation-induced damage [11]; treatments to target overt inflammatory responses have shown clinical benefits [15]. Phosphodiesterase inhibitors [16,17] with anti-TNFα effects have been tested in safety trials and drugs such as etanercept or infliximab were effective as adjunctive therapy in animal models [18]. The overall efficacy of prednisolone and dexamethasone resulted in improved survival in TB meningitis patients (17% reduction in mortality across 41 clinical trials) [15].

MSC can be involved in other important immune mechanisms: MSC stimulation with inflammatory cytokines has induced a broad range of antimicrobial effector functions mediated by the

Table 3
Number and percentage of patients with a successful outcome (cured or completed treatment at first recording of an outcome) stratified by potential confounders.

Variable	Cases ($n = 36$)	Study controls ($n = 36$)	p-Value* comparing cases with study controls	Surveillance controls ($n = 36$)	p-Value* comparing cases with surveillance controls
Age					
< 35 years old	20/25 (80.0%)	8/17 (47.1%)	0.045	3/8 (37.5%)	0.036
35 years or older	9/11 (81.8%)	6/19 (31.6%)	0.021	12/28 (42.9%)	0.038
Gender					
Males	13/18 (72.2%)	4/11 (36.4%)	0.12	15/31 (48.4%)	0.14
Females	16/18 (88.9%)	10/25 (40.0%)	0.002	0/5 (0.0%)	0.006
Treatment history					
New case	13/13 (100.0%)	8/19 (42.1%)	0.001	6/13 (46.2%)	0.005
Previously treated case	10/11 (80.9%)	4/13 (30.8%)	0.005	9/23 (39.1%)	0.075
Chronic case	6/12 (50.0%)	2/4 (50.0%)	>0.99		
Drug resistance profile					
MDR	9/9 (100.0%)	9/20 (45.0%)	0.005	3/9 (33.3%)	0.009
Pre-XDR	9/12 (75.0%)	5/10 (50.0%)	0.38	8/12 (66.7%)	>0.99
XDR	11/15 (73.3%)	0/6 (0.0%)	0.004	4/15 (26.7%)	0.027
Smear status at start of chemotherapy					
Positive	3/5 (60.0%)	5/18 (27.8%)	0.30	2/5 (40.0%)	>0.99
Negative	26/31 (83.9%)	9/18 (50.0%)	0.020	13/31 (41.9%)	0.001
Current smoker					
Yes	15/20 (75.0%)	10/25 (40.0%)	0.034	10/26 (38.5%)	0.019
No	14/16 (87.5%)	4/11 (36.4%)	0.012	5/10 (50.0%)	0.069
Employment status					
Unemployed	4/5 (80.0%)	2/13 (15.4%)	0.022	1/3 (33.3%)	0.49
Employed	16/18 (88.9%)	5/13 (38.5%)	0.006	11/23 (47.8%)	0.008
On disability benefits	3/6 (50.0%)	2/4 (50.0%)	>0.99	2/7 (28.6%)	0.59
Student	4/5 (80.0%)	1/1 (100.0%)	>0.99	0/0	N/A
Maternity leave/housewife	2/2 (100.0%)	1/1 (100.0%)	>0.99	0/1 (0.0%)	0.33
Retired	0/0	3/4 (75.0%)	N/A	1/2 (50.0%)	N/A
Marital status					
Single	12/17 (70.6%)	8/20 (40.0%)	0.10	8/16 (50.0%)	0.30
Married	16/18 (88.9%)	5/13 (38.5%)	0.006	7/16 (43.8%)	0.009
Divorced	1/1 (100.0%)	1/2 (50.0%)	>0.99	0/4 (0.0%)	0.20
Widowed	0/0	0/1 (0.0%)	N/A	0/0	N/A
Highest education level					
Secondary school	5/9 (55.6%)	4/11 (36.4%)	0.65	2/5 (40.0%)	>0.99
College	15/17 (88.2%)	7/17 (41.2%)	0.010	8/22 (36.4%)	0.001
Currently at university	4/5 (80.0%)	1/1 (100.0%)	>0.99	0/0	N/A
University	5/5 (100.0%)	1/2 (50.0%)	0.29	5/9 (55.6%)	0.22
No data	0/0	1/5 (20.0%)	N/A	0/0	N/A

* p-Values show the result of Fisher's exact test, testing the null hypothesis that successful outcome rates are equal between cases and controls. A p-Value less than 0.05 indicates evidence of a statistically significant difference in the percentages of cases and control with successful outcomes.

Table 4
Results of univariable and bivariable conditional logistic regression modeling the odds of a successful outcome. The control group used here are the surveillance controls 1:1 matched to cases on (a) drug resistance profile, (b) smear status at start of chemotherapy and (c) previous treatment status. Differences in the odds ratios illustrate the confounding effects of each variable on the estimated odds ratio of the MSC treatment on outcomes.

Model	Variable	Odds ratio (95% CI)	p-Value
Univariable	Case versus control	7.77 (1.78, 33.81)	0.006
Bivariable: adjusting for age[a]	Case versus control	6.51 (1.17, 36.22)	0.032
	Age, for each additional year	0.98 (0.90, 1.08)	0.73
Bivariable: adjusting for gender	Case versus control	12.54 (1.61, 97.91)	0.016
	Gender, male versus female	2.86 (0.27, 30.31)	0.38
Bivariable: adjusting for current smoker	Case versus control	10.37 (1.53, 70.07)	0.016
	Current smoker, yes versus no	0.17 (0.01, 2.54)	0.20
Bivariable: adjusting for employment status	Case versus control	7.76 (1.78, 33.78)	0.006
	Employed versus all other categories[b]	0.95 (0.13, 6.95)	0.96
Bivariable: adjusting for marital status	Case versus control	12.29 (1.68, 89.78)	0.013
	Married versus single/divorced	0.24 (0.02, 2.54)	0.24
	Case versus control	11.26 (1.61, 78.95)	0.015
Bivariable: adjusting for education level	College/university versus secondary school	3.37 (0.22, 50.93)	0.38

[a] Age was entered into the model as a continuous variable.
[b] Categories for employment status included in "other" were: unemployed, on disability benefits, student, on maternity leave/housewife and retired.

tryptophan catabolising enzyme indoleamine 2,3-dioxygenase [19]; MSCs act by reduction of oxidative stress, which was shown to be operational in a murine model of acute coxsackievirus B3-induced myocarditis [20]; enhanced phagocytosis was, in part, responsible for increased bacterial clearance and improved survival in a murine sepsis model [21] and finally, MSC possess direct antimicrobial activity, which is mediated by the secretion of human cathelicidin hCAP-18/ LL-37 [22].

Several studies have shown that MSCs exhibited therapeutic potential in preclinical models of acute lung injury [23], endotoxin- [24] and bleomycin-induced lung injury [25], associated with decreased expression of transforming growth factor $\beta 1$ responsible for pulmonary fibrosis [26]. In addition, lung tissue regeneration capacity of MSC has been shown previously [27,28]. There have been other in vivo studies of MSC for lung disease. For example, the START trial was a Phase I trial of intravenous MSCs in patients with Acute Respiratory Distress Syndrome (ARDS). This study showed that MSCs were well-tolerated in the nine patients and the authors have now proceeded to Phase II testing [29]. There was another, separate, pilot study of MSCs in patients with ARDS that also found that they were well-tolerated, although the clinical effect was weak [30]. MSCs have also been studied in patients with Chronic Obstructive Pulmonary Disease (COPD) and have also been shown to be well-tolerated in these patients [31].

MSC can be engrafted into the lungs and differentiated into various types of lung cells: alveolar type I and type II cells [27,32] airway epithelium cells [33,34] and endothelial cells [32]. In addition, MSC can restore lung epithelium via donation of mitochondria to other cells [33] and increase the proliferative potential of bronchoalveolar stem cells [34]. In animal model experiments a large fraction of systemically (intravenously) infused MSC typically become trapped within the lungs owing to their large size and their repertoire of cell-surface adhesion receptors [35–38]. Real-time PCR analysis for human-specific Alu sequences in blood samples showed that within 5 min of MSC infusion through the tail vein, 99% of MSC were cleared from the circulation. Within 10–30 min, a resurgence of ~2–3% of the infused MSC was observed within the blood stream. Tissue samples from various organs revealed that the majority of cells were initially found in the lung, which is consistent with previous studies. Then, 15 min after infusion, 83% of the human DNA was detected in the lung, whereas only trace amounts were detected in other tissues [39]. Plate-adherent cultured bone marrow cells (i.e. MSCs), when given intravenously in wild-type mice following bleomycin-induced lung injury, engrafted into the recipient lung parenchyma with a morphological and molecular phenotype of alveolar type I pneumocytes [27]. Human umbilical cord MSC when cultured in vitro with specialized growth medium/growth factors expressed Clara cell secretory protein (CCSP), surfactant protein C (SPC) and cystic fibrosis transmembrane conductance regulator (CFTR). After systemic administration to immunotolerant, NOD-SCID mice, cells were localized in the lung airway epithelium that expressed cytokeratin and human CFTR [28]. Wong et al. found a subpopulation of adherent human and murine bone marrow cells (currently called MSC) that expressed CCSP, and when cultured ex vivo with an air-liquid interface, these CCSP+ cells expressed alveolar type I and II markers such as pro-SPC, CFTR and epithelial sodium channel (ENaC). CCSP+ cells preferentially homed to naphthalene-damaged airways when delivered transtracheally or intravenously [40,41].

Our study has some limitations. When comparing cases to study controls, the patient recruitment method could have biased the resulting efficacy estimate. Patients choosing the treatment may be exceptionally motivated to be cured and thus more likely to have a positive outcome than a randomly selected MDR-TB patient. This motivation may be reflected in their lifestyle choices and their adherence to chemotherapy, which could influence their outcome. To account for this, we sought out additional information as a proxy for these factors and analysis adjusting for these data showed no change in the overall conclusion (Appendix Table S4). The treatment effect was maintained when comparing with matched controls from the Belarussian surveillance data, even after adjusting for the only identifiable negative confounder (Table 4). Therefore, it seems unlikely that confounding factors can fully explain the observed treatment effect. However, our results may not be generalizable to patients with different characteristics from those studied here. In particular, there were very low numbers of cases with smear-positive disease; further studies of MSC treatment should ensure recruitment of sufficient numbers of smear-positive individuals.

Other unmeasureable baseline differences between cases and surveillance controls might explain the observed treatment effect. However, a greater proportion of surveillance controls had converted to culture negative status at two months after chemotherapy start than cases. At this point, some MSC patients had yet to receive the infusion and many had only very recently received it (average receipt of infusion was 49 days after chemotherapy start). Therefore, given the documented association between two-month culture conversion and successful treatment outcome [42,43], we would expect the cases to experience poorer outcomes than the surveillance controls. However, at four and six months, having all received the infusion some time previously, there were very similar proportions of cases that were culture negative as among surveillance controls and the cases experienced better outcomes. This pattern was consistent when comparing cases to study controls. These data are consistent with the hypothesis that the baseline characteristics of the cases cannot fully explain the improved outcomes observed in this group and instead something external (e.g. the MSC treatment) occurred to change the trajectory of these patients. In addition, radiology analysis showed that significantly more of the cases showed improvements than study controls.

Our study may have been under-powered to detect a difference in adverse events between the groups. Further studies should be sufficiently powered and randomized to identify any increase in adverse events experienced by MSC recipients.

The current treatment situation for M/XDR-TB cases is dire. Only 20% of the estimated global incident MDR-TB cases in 2013 were successfully diagnosed and started on appropriate treatment [1]. Although some countries have documented relatively high rates of successful outcomes [44] (around 70%), many countries successfully treat less than half of their treated M/XDR-TB cases [4]. Our results demonstrate that MSC could revolutionize outcomes for individuals with MDR-TB. With patients dying from M/XDR-TB daily, randomized, controlled trials of MSC infusion are urgently needed to confirm these findings so that patients can start benefitting from this novel treatment.

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